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<b>(54) Title:</b> HEPATOSELECTIVE PHARMACEUTICAL ACTIVES			
<b>(57) Abstract</b> <p>The invention provides an analogue of a pharmaceutical active whose molecular weight is less than 25,000 Daltons, the analogue comprising a pharmaceutical active whose molecular weight is less than 25,000 Daltons covalently linked to a pendant molecular group wherein as a result of the administration of the composition to the human or animal body an active complex having a molecular weight of 25,000 Daltons or greater is present in the human or animal circulatory system. The analogue is hepatoselective when administered to the circulatory system. Preferably the analogue is an insulin analogue comprising an insulin or functional equivalent thereof covalently linked to the pendant molecular group wherein the active complex is an insulin complex. Such an insulin analogue may be used in a method of insulin replacement therapy.</p>			

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HEPATOSELECTIVE PHARMACEUTICAL ACTIVES

The present invention relates to novel hepatoselective pharmaceutical actives. In particular it relates to novel  
5 hepatoselective insulin analogues suitable for use in an improved treatment of diabetes mellitus.

When drugs and other pharmaceutical actives are administered to the human or animal body it may be required that the active is needed to be present primarily in the  
10 liver or to act primarily on the tissues of the liver. That is, the active is required to be hepatoselective. Achieving hepatoselectivity can be difficult, in particular where the active is administered by injection into the skin. One case in which achievement of hepatoselectivity  
15 would be especially desirable is the administration of insulin.

The hormone insulin, secreted by the pancreas, has various important roles to play in glucose metabolism. In the liver, after binding to cell surface receptors, insulin  
20 promotes the conversion of glucose to glycogen (glycogenesis), promotes protein synthesis and inhibits fat breakdown. Insulin deficiency results in breakdown of glycogen (glycogenolysis), protein and conversion of products of fat and protein breakdown to glucose  
25 (gluconeogenesis) leading to a raised plasma glucose level (hyperglycaemia). In subjects who produce adequate amounts of insulin the blood glucose level remains within a certain range. Any excess of glucose is stored in the liver and muscles as glycogen.

30 Insulin also acts on cell membrane receptors in other tissues to enhance the entry of glucose into cells, thereby diminishing the plasma glucose concentration.

Thus insulin acts to reduce the plasma glucose level by reducing the production of glucose by the liver and by  
35 increasing uptake and metabolism of glucose by the liver and by increasing uptake and metabolism of glucose by peripheral tissues.

Deficiency of insulin due to disease of the islets of Langerhans and/or deficiency of insulin action results in diabetes mellitus, a condition in which the blood glucose concentration is high.

5 In subjects who are not diabetic insulin is produced in the pancreas and transported directly to the hepatic circulation and hence is transported to the liver before any other organs or regions of the body. Thus the liver experiences a very high exposure to the insulin produced.  
10 Usually at least 50% of the insulin produced is bound to receptors in the liver, and hence acts in the liver. Insulin bound to receptors in the liver is removed from the circulation and degraded by the liver cells. The insulin which is not bound in the liver and hence passes to the  
15 peripheral circulation is therefore at a much lower concentration. Thus the peripheral tissues (eg fat and muscle) which are also targets of the insulin experience a smaller exposure to the insulin secreted.

In diabetic subjects treatment is often carried out by  
20 insulin-replacement therapy. In general an insulin preparation is injected subcutaneously. The most common subcutaneous insulin regimen involves twice-daily injection of mixtures of short- and intermediate-acting insulin preparations. Insulin is absorbed from the subcutis into  
25 the peripheral circulation and thence to the entire body, including the liver. With such a system the liver and the peripheral tissues tend to experience approximately equal exposure to insulin.

There are various disadvantages and negative side-  
30 effects with the use of this system.

First, if sufficient insulin is injected to enable a high enough concentration to be present in the hepatic circulation then too high a concentration will be present in the peripheral circulation. Similarly if a suitable  
35 concentration is present in the peripheral circulation the concentration of insulin in the hepatic circulation will be inadequate.

There is believed to be a danger associated with high concentrations of insulin in the peripheral circulation (hyperinsulinaemia) of cardiovascular disease.

5 Second, there is a serious risk of hypoglycaemia in diabetic subjects receiving insulin replacement therapy by subcutaneous injection. A concentration of insulin in the peripheral circulation which is too high can lead to a blood sugar level which is too low and subsequent collapse.

10 It would therefore be desirable to be able to direct a large concentration of insulin directly to the hepatic insulin receptors with a lower concentration of insulin being directed to the peripheral insulin receptors. It would also be desirable to achieve such hepatoselectivity for other active molecules.

15 Some attempts have been made to obtain the desired hepatoselectivity of insulin. Intravenous injection directly into the hepatic portal circulation could allow injected insulin to pass directly to the liver before reaching the peripheral circulation. Such a system is  
20 unsuitable for general use due to the difficulty and complicated nature of its operation; in particular it is not suitable for use by diabetic patients themselves except under exceptional circumstances.

An attempt has been made to render insulin  
25 hepatoselective by injecting it subcutaneously whilst encapsulated in lipid vesicles. (Spangler, Ronald S., "Selective Insulinisation of Liver in Conscious Diabetic Dogs", Am. J. Physiol. 249 (Endocrinol. Metab. 12) : E152 - E159, 1985). Insulin encapsulated in lipid vesicles  
30 (designated vesicle encapsulated insulin VEI) was targeted to hepatocytes by means of a digalactosyl diglyceride moiety incorporated on the outside of the lipid vesicles. Using this approach it was possible to alter the distribution of an administered glucose load to favour  
35 hepatic deposition.

The present inventors have found (British Diabetic Association Medical and Scientific section Conference,

Manchester, 13-15 April 1989) that covalent dimers of insulin (molecular weight  $\pm 12,000$  Daltons rather than  $\pm 6,000$  for insulin monomer) have a greater effect on hepatic glucose output than on peripheral uptake and utilisation. That is, the covalent insulin dimers appear to act preferentially on hepatocytes rather than on cells of the peripheral tissues. It has also been found that proinsulin shows this hepatoselectivity to a smaller degree. Proinsulin is the zymogen which is cleaved to form the active hormone insulin. The proinsulin molecule is larger than the active insulin molecule.

The cells of the peripheral tissues, for instance fat and muscle, are separated from the blood vessels by the capillary endothelium. In the liver however there is no such barrier between blood vessels and hepatocytes.

It is thought that transport across the capillary endothelium is mainly by diffusion i.e. active transport of insulin across the capillary endothelium does not occur to any significant extent. Therefore the present inventors believe that the absorption of insulin into the peripheral tissues is determined by factors influencing diffusive transport, in particular by steric hindrance or size of molecules. This is believed to be a reason for the relative hepatoselectivity of covalent insulin dimers and proinsulin when compared with insulin monomers; both have free access to hepatocytes but the larger covalent insulin dimer or proinsulin is absorbed into the peripheral tissues from the bloodstream more slowly than is the insulin monomer. Thus the larger molecules spend a longer time in the bloodstream before being absorbed into the peripheral tissues and are thus more likely to reach the liver, where they may be active more easily.

According to the present invention there is provided the use of an analogue of a pharmaceutical active whose molecular weight is less than 25,000 Daltons in the manufacture of a composition for use in a method of treatment of the human or animal body, the analogue

comprising a pharmaceutical active whose molecular weight is less than 25,000 Daltons covalently linked to a pendant molecular group wherein as a result of the administration of the composition to the human or animal body an active  
5 complex having a molecular weight of 25,000 Daltons or greater is present in the human or animal circulatory system.

The invention is of particular usefulness when the analogue is an insulin analogue which comprises an insulin  
10 or functional equivalent thereof covalently linked to a pendant molecular group, so that as a result of the administration of the composition to the human or animal body an insulin complex having a molecular weight of 25,000 Daltons or greater is present in the human or animal  
15 circulatory system. This invention is also applicable to other pharmaceutical actives of molecular weight below 25,000 Daltons which are administered so as to enter the circulatory system and which are required to be hepatoselective and for which similar problems as those  
20 encountered with the administration of insulin therefore also apply. In this specification the invention is discussed primarily in terms of its use for giving hepatoselectivity to insulin or a functional equivalent thereof, but it will be understood that the disclosures  
25 made are equally applicable to other pharmaceutical actives of molecular weight less than 25,000 Daltons.

According to a first, particularly preferred, embodiment of the invention the pendant molecular group has an affinity for one or more binding proteins present in the  
30 human or animal circulatory system wherein the total molecular weight of the insulin or functional equivalent thereof (or other pharmaceutical active) and the additional molecular group and the one or more binding proteins is 25,000 Daltons or greater.

35 Binding proteins "in the human or animal circulatory system" are those which are present in the blood plasma.

Thus when the analogue is introduced into the bloodstream the one or more binding proteins will become non-covalently linked to the additional molecular group, forming a complex which has a molecular weight of 25,000  
5 Daltons or greater.

According to a second embodiment of the invention an insulin analogue (or analogue of another pharmaceutical active) is used which additionally comprises one or more binding proteins non-covalently attached to the pendant  
10 molecular group wherein the total molecular weight of the insulin or functional equivalent thereof (or other pharmaceutical active) and the pendant molecular group and the one or more binding proteins is 25,000 or greater.

In this second embodiment the analogue is a complex  
15 having a molecular weight of 25,000 or greater and may be administered into the circulatory system as such, for instance intravenously.

According to a third embodiment of the invention an insulin analogue (or analogue of other pharmaceutical active) is used in which the total molecular weight of the  
20 insulin or functional equivalent thereof (or other pharmaceutical active) and the covalently linked pendant molecular group is 25,000 Daltons or greater.

As with the second embodiment, the analogue is a  
25 complex having a molecular weight of 25,000 or greater and may be administered into the circulatory system as such, for instance intravenously.

For use in the first aspect of the present invention there is provided an analogue of a pharmaceutical active whose molecular weight is less than 25,000 Daltons  
30 comprising a pharmaceutical active whose molecular weight is less than 25,000 Daltons covalently linked to a pendant molecular group, said pendant molecular group having an affinity for one or more binding proteins present in the  
35 human or animal circulatory system.

As explained above, the invention is especially useful when the compound is an insulin analogue comprising an



insulin or functional equivalent thereof covalently linked to a pendant molecular group which has an affinity for one or more binding proteins present in the human or animal circulatory system.

5        Such an insulin analogue (or analogue of other pharmaceutical active) may be injected subcutaneously and absorbed into the bloodstream through the capillary endothelium without difficulty. When in the bloodstream the insulin analogue will come into contact with the  
10 binding protein for which the covalently-linked pendant molecular group has an affinity. Thus at least some molecules of the insulin analogue will become bound to the said binding protein, forming an insulin complex. Binding proteins tend to be bulky molecules of high molecular  
15 weight. They therefore tend not to diffuse out through the capillary endothelium easily and remain in the bloodstream. Thus the effective size of molecule and hence the molecular weight of the bound insulin analogue is increased dramatically. Absorption from the blood vessels into the  
20 peripheral tissues, for instance fat and muscle, through the capillary endothelium is now greatly inhibited due to the attachment of the insulin analogue to the high molecular weight binding protein. In the liver however there is no such barrier therefore the insulin analogue  
25 even with the associated binding protein may have access to the hepatocyte insulin receptors essentially to the same degree as would conventional insulin.

In the same way an analogue of another pharmaceutical active may be induced not to diffuse out through the  
30 capillary endothelium and to remain in the bloodstream until it is carried to the liver where it may act or be taken up as required.

Binding of the pendant molecular group and the binding protein is not covalent. Binding forces may be for  
35 instance electrostatic (eg attraction of opposite charges, hydrogen bonding) or hydrophobic. Thus binding is not permanent. The insulin analogue (or analogue of other

pharmaceutical active) may be absorbed onto the hepatic tissues in its bound form. Molecules of the insulin analogue which have not been bound by the binding protein or which have become bound and subsequently unbound are  
5 capable of passing through the capillary endothelium into the peripheral tissues.

The covalently-linked molecular group is attached in such a way that the active site (or sites) of the insulin equivalent or other pharmaceutical active remains available  
10 to carry out its prescribed functions.

Attachment of a suitable molecular group to the insulin equivalent (or other active) may be carried out by conventional chemical methods known to those skilled in the art.

15 According to this aspect of the invention there is also provided a method of insulin replacement therapy comprising subcutaneous injection of a preparation comprising an insulin analogue as described above.

Preferred features of the first embodiment of the  
20 invention will now be described in detail.

The insulin or functional equivalent thereof, when insulin is the active used, may be any of the insulins conventionally used in insulin replacement therapy.

J. Brange et al ("Monomeric Insulins and their  
25 Experimental and Clinical Implications", Diabetes Care, vol. 13, no. 9, September 1990) and others have studied the possibility of developing insulins with reduced tendencies to self-association. These insulins are absorbed from the subcutis into the bloodstream more rapidly than is the form  
30 in which insulin is usually found in pharmaceutical formulations. Insulin assumes an associated state in pharmaceutical formulation. Six monomers of insulin associate to form hexamers. The association is non-covalent. With the use of DNA technology Brange et al and  
35 others have prepared insulins which remain dimeric or even monomeric at high (pharmaceutical) concentration by the introduction of one or a few amino acid substitutions into

human insulin. Insulins with reduced association capacity as described by Brange et al and others are preferred for use as the insulin equivalent to which the additional molecular group is attached. When the pharmaceutical  
5 active is an insulin equivalent such insulins are preferred, because their reduced tendency to self-associate means that they are more rapidly absorbed into the bloodstream from the subcutis than are conventional insulins which tend to be injected in hexameric form.

10 Insulins of this type have also been developed by Eli Lilly. These are described in Protein Engineering, Vol 5, 519-525 and 527-533 (1992) (both D. N. Brems et al). Studies of amino acid modified monomeric insulins have also been described in Diabetes 40 Suppl. 1 (1991), 423A (Howey  
15 et al) and 464A (Shaw et al).

The pendant molecular group covalently linked to the insulin equivalent or other active may be any molecular group which has an affinity for a binding protein present in the circulatory system and which will not itself act in  
20 the body to give detrimental effects. The molecular group chosen should usually be of a molecular weight similar to or less than that of the insulin equivalent or other active. Preferably the total molecular weight of the insulin or functional equivalent thereof or other active  
25 and the additional molecular group is less than 25,000, more preferably less than 20,000 or 15,000, and may be less than 12,000. This is in order that attachment of the pendant molecular group to the insulin equivalent or other active to form the analogue should not hinder the passage  
30 by diffusion of the injected analogue from the subcutis through the capillary endothelium into the bloodstream. Preferred molecular groups are molecular structures which possess an affinity for one or more proteins which are naturally present in the circulation. They may be based on  
35 naturally-occurring hormones or on functional equivalents of such hormones which also possess affinity to their binding proteins or they may be based on other substances

for which such binding proteins exist. The pendant molecular group should be harmless when injected into the body; this may be achieved for instance by ensuring that the concentration of insulin analogue or analogue of other  
5 active in the bloodstream is high enough to allow the beneficial effects of insulin or other therapy to be felt but low enough to prevent any effects which might be due to the pendant molecular group being felt, or by ensuring that the insulin analogue or analogue of other active is not  
10 present in parts of the body where the pendant molecular group might be active, or by rendering the pendant molecular group inactive by structural modification which nevertheless preserves its ability to bind to its binding protein.

15 The pendant molecular group may be itself an insulin equivalent, for instance insulin-like growth factor 1 (IGF1). This polypeptide has an affinity for IGF1 binding proteins, which circulate naturally in the human bloodstream.

20 The pendant molecular group may be a native or modified thyroxyl group, derived from the human thyroid hormone thyroxine 3,5,3',5'-L-tetraiodothyronine (T4). There are several binding proteins present in the human circulatory system which have an affinity for the T4 group,  
25 for instance thyroxine binding globulin (TBG), thyroxine binding prealbumin (TBPA) and albumin. These proteins are known collectively as thyroxine binding proteins (TBP).

Other suitable groups derived from hormones or their functional equivalents may be used. Suitable groups may be  
30 ascertained by a skilled person using methods known in the pharmaceutical field.

The pendant molecular group with an affinity for a binding protein may be covalently attached directly to the insulin equivalent (or other active), as explained above in  
35 a place chosen so that the additional molecular group does not inhibit the action of the insulin equivalent. The structure of insulin and the locations of its active sites

are well known, therefore when insulin is used those skilled in the art will be able to establish appropriate positions at which to attach the said molecular group so as not to interfere significantly with the action of the insulin or equivalent.

Alternatively a short molecular chain (or "spacer arm") may be used to link the insulin equivalent (or other active) and the said molecular group. The spacer arm is linked covalently both to the insulin equivalent and to the pendant molecular group. Such a spacer arm ensures that the insulin equivalent and binding protein are distanced from one another, thus preventing substantial interference with insulin activity by the (usually bulky) high molecular weight binding protein. The spacer arm is usually a linear chain, preferably of from 3-10 carbon atoms in length. Such a spacer arm may be for instance an aminohexanoyl (AH) group, which is a six-carbon chain. Other groups may of course be used as a spacer arm. For example aminoacids either singly or linked as small peptide sequences could be used.

It should be ensured that the binding protein which has an affinity for the pendant molecular group is present in the blood plasma in sufficiently large amounts that the trapping of the binding protein by the insulin analogue (or analogue of other active) with the covalently linked molecular group will not deplete the levels of binding protein in the blood to detrimental effect. For instance the level of binding protein should not be depleted so that insufficient binding protein is available to carry out its usual function in the body, if any.

The insulin analogue (or analogue of other active) of the invention may be produced by various methods, for instance: chemical reaction of the insulin or insulin equivalent or other active with a substance or substances which include the pendant molecular group; protein synthesis of the complete analogue; production by a genetically engineered micro organism.

The insulin analogue of the invention may be used in a method of insulin replacement therapy.

An insulin analogue according to the invention or a mixture of two or more different insulin analogues according to the invention form part of an insulin preparation. This insulin preparation is suitable for use in a method of treatment of the human or animal body, preferably suitable for subcutaneous injection, and may comprise a treatment for diabetes. Additional ingredients may be added which modify the rate of absorption from the subcutaneous depot into the circulation. The insulin-based preparation preferably is suitable for subcutaneous injection, in which case it is therefore suitable for use by sufferers from diabetes on themselves.

The insulin preparation may comprise the hepatoselective insulin analogue of the invention and a conventional non-hepatoselective insulin. On subcutaneous injection and passing into the bloodstream the conventional insulin will act in the peripheral tissues whilst the insulin analogue of the invention provides a controlled, hepatoselective action.

The analogues of other pharmaceutical actives of the invention may also be used in methods of treatment or therapy by subcutaneous injection of a preparation comprising the analogue of the active.

According to the second embodiment of the invention an insulin analogue (or analogue of other active) with a covalently attached pendant molecular group is used with a binding protein already non-covalently linked to the pendant molecular group. A composition comprising such an insulin analogue would be more suitable for intravenous administration than for subcutaneous administration due to the very high molecular weight of the insulin analogue in the composition (as compared with the insulin analogue of the first embodiment, which forms an insulin complex of molecular weight greater than 25,000 only after administration into the circulatory system). It may be

desirable in some cases however to provide such an insulin analogue or analogue of other active for use in the manufacture of a composition suitable for intravenous administration.

5       The pendant molecular group and binding proteins may be any of those described above as suitable for the first embodiment of the invention. In addition the binding proteins may be proteins which do not occur naturally in the human or animal circulatory system (and which are  
10       harmless when introduced into the circulatory system) and the pendant molecular group may be any group with an affinity to such a protein.

      According to a third embodiment of the invention an insulin analogue (or analogue of other active) is used  
15       which comprises an insulin or functional equivalent thereof (or other active) with a large group covalently attached. Such a group should be large enough that the entire insulin analogue has a molecular weight of at least 25,000. It should also, like the pendant molecular group of the first  
20       embodiment of the invention, be harmless when introduced into the body. This may be achieved as described above for the pendant molecular group. The large molecular group of the third embodiment of the invention may for instance be based on a polypeptide structure or on other suitable  
25       polymeric structures.

      The analogues of the second and third embodiments of the invention may be produced by the methods mentioned above as suitable for the production of the analogue of the first embodiment of the invention. For instance suitable  
30       methods in the case of the analogue of the third embodiment include chemical reaction of the insulin or equivalent or other active with a substance or substances which include the covalently linked molecular group, protein synthesis of the complete analogue and production by a genetically  
35       engineered micro organism. In the case of the second embodiment the section of the analogue comprising the insulin or equivalent or other active and the additional

molecular group may be synthesized in the same way as may be the analogue of the first embodiment. The binding protein may be synthesized for instance by protein synthesis, production by a genetically engineered micro organism, extraction from a naturally-occurring organism. Non-covalent attachment of the binding protein or proteins to the pendant molecular group may be achieved by methods known in the art.

The analogues of the second and third aspects of the invention are suitable for incorporation in compositions which are to be administered intravenously. Where the active is insulin or a functional equivalent thereof, such compositions may be used in a method of insulin replacement therapy. Such compositions comprise one or more insulin analogues of the second and third embodiments of the invention. The composition may also comprise additional ingredients which may be chosen by those skilled in the art.

#### Examples

The present inventors have undertaken a study to explore the possibility that insulin analogues with restricted access to peripheral tissues may display relative hepatoselectivity in vivo.

Analogues of insulin which include a thyroxyl moiety which binds to thyroid hormone binding protein (TBP) have been designed and tested. N<sup>αB1</sup>-thyroxyl-insulin (T4-Ins) and N<sup>αB1</sup>-thyroxyl-aminohexanoyl insulin (T4-AH-Ins) were synthesized using methods of chemical synthesis given below.

#### Preparation of thyroxyl insulins

Abbreviations: Msc = methylsulphonylethyloxycarbonyl

Boc = tert. butyloxycarbonyl

DMF = dimethylformamide

DMSO = dimethylsulfoxide

mp = melting point

ONSU = N-oxysuccinimide ester

#### EXAMPLE 1



B1-thyroxyl-insulin (porcine) (T4-Ins)

Protected thyroxine derivatives:

Msc-L-thyroxine (I)

776 mg (1 mmol) L-thyroxine in 2 ml dimethylsulfoxide  
5 was reacted with 530 mg (2 mmol) Msc-ONSu in the presence  
of 139  $\mu$ l (1 mmol) triethylamine at room temperature for 18  
hours. After concentration in vacuo the oily residue was  
taken up in ethyl acetate, the organic layer washed with  
water, dried over Na<sub>2</sub>SO<sub>4</sub>, and the solvent removed in vacuo.  
10 The solid residue was crystallised from hexane.  
Yield: 651 mg (70.2% of theory),  
mp: 200°C

Msc-L-thyroxine-N-oxysuccinimide ester (II)

To a cooled (0°C) solution of 371 mg (0.4 mmol) of Msc-  
15 thyroxine and 40 mg (0.4 mmol) N-hydroxysuccinimide in 1 ml  
tetrahydrofuran a precooled solution of 82.5 mg N,N'-  
dicyclohexycarbodiimide in 0.5 ml tetrahydrofuran was  
added under stirring. After further stirring for 3 hours  
the precipitate was removed by filtration, and the filtrate  
20 concentrated in vacuo. Crystallisation of the solid  
residue from methylenechloride/petroleum ether.  
Yield: 295 mg (71%, based on I)  
mp: 180°C.

B1-thyroxyl-insulin (porcine) (III)

25 To a solution of 100 mg (approx 0.016 mmol) A1,B29-  
Msc<sub>2</sub>-insulin (prepared according to Schüttler and  
Brandenburg, Hoppe-Seyler's Z. Physiol, Chem. 360, 1721 -  
1725 (1979)) and 18  $\mu$ l (0.160 mmol) N-methylmorpholine in 2  
ml of DMSO 116.4 ml (0.160 mmol) of II in 0.2 ml DMSO were  
30 added. After stirring for 6 h at room temperature the  
insulin derivative was precipitated with ether/methanol  
(9:1, v/v), isolated by centrifugation, washed 3 times with  
ether/methanol, and dried in vacuo.

Msc groups were removed by treatment with  
35 NaOH/dioxane/methanol at 0°C, and III was purified by gel  
filtration on Sephadex G 50 fine as described (Geiger et  
al, Chem. Ber. 108, 2758 - 2763 (1975)). Lyophilization

gave 78.4 mg B1-thyroxyl-insulin (75%, based on Msc<sub>2</sub>-insulin).

#### EXAMPLE 2

##### B1-thyroxyl-aminohexanol-insulin (porcine) (IV) (T4-AH-Ins)

- 5 1. BOC- $\epsilon$ -aminohexanoic acid was prepared by reacting 1.32 g (10 mmol)  $\epsilon$ -aminohexanoic acid with 2.4 g (11 mmol) di-tert.butylidicarbonate in dioxane/water at pH 9 and was obtained in 87% yield (2.2 g). Mp after recrystallisation from ethyl acetate: 75°C.
- 10 2. 22.8 mg (0.096 mmol) Boc-aminohexanoic acid was preactivated with 13 mg 1-hydroxybenzotriazole and 17.8 mg (0.09 mmol) dicyclohexyl carbodiimide in 0.7 ml dimethylformamide for 1 h at 0°C and 1 further hour at room temperature.
- 15 3. Then, a solution of 100 mg (approx. 0.016 mmol) A1,B29-Msc<sub>2</sub>-insulin and 18 $\mu$ l (0.160mmol) N-methylmorpholine in 1 ml of DMF was added. After stirring for 70 minutes at room temperature the mixtures was filtered, and the insulin derivative was precipitated with ethyl ether/methanol (9:1,
- 20 v/v), isolated by centrifugation, washed 3 times with ether/methanol, and dried in vacuo.
4. The Boc protecting group was cleaved by treatment of the product with 3 ml trifluoro acetic acid for 1 hour at room temperature. The solution was concentrated in vacuo,
- 25 the insulin derivative precipitated with ether, isolated, washed with ether, and dried. Yield: 77.8 mg of B1-aminohexyl-A1,B29-Msc<sub>2</sub>-insulin.
5. 102 mg (0.016 mmol) of this derivative was dissolved in 2 ml dimethylformamide and 18 $\mu$ l (0.16 mmol) N-
- 30 methylmorpholine. After addition of 116 mg (0.16 mmol) Msc-thyroxine-N-oxysuccinimide ester in 0.2 ml dimethylformamide the mixture was stirred for 3 hours at room temperature. The protected insulin was isolated by precipitation with methanol/ether and isolated as described
- 35 above.
6. Msc groups were removed by treatment with NaOH/dioxane/methanol at 0°C, and IV was purified by gel

filtration on Sephadex G 50 fine as described (Geiger et al, op. cit.). Lyophilization gave 39.1 mg B1-thyroxyl-aminohexanoyl-insulin (37%, based on aminohexyl-Msc<sub>2</sub>-insulin).

5           T4-Ins, T4-AH-Ins and insulin (Ins) were infused into four anaesthetized beagles with D-<sup>3</sup>H-3-glucose for measurement of the rates of glucose production (Ra) and glucose disposal (Rd). Euglycaemia and glucose specific activity were maintained by variable infusion of D-glucose  
10       with D-<sup>3</sup>H-3-glucose.

          With all three materials glucose Rd was increased and glucose Ra decreased from basal level  $2.70 \pm 0.19 \text{ mg. kg}^{-1} \text{ min}^{-1}$ , ( $p < 0.05$ ). In each experiment insulin-like activity for Ra and Rd was calculated as the area between the basal  
15       values of each of these variables and subsequent values plotted graphically against time (AUC). For Ins, T4-Ins and T4-AH-Ins respectively, AUC for Ra values were  $-431 \pm 121$ ,  $-226 \pm 154$  and  $-357 \pm 50$  (mean  $\pm$  SEM, mg/kg), (no significant differences) and AUC for Rd values were  
20        $1142 \pm 160$ ,  $629 \pm 125$  and  $830 \pm 178$  mg/kg, both analogues different from Ins  $p < 0.05$ .

          These results indicate that insulin analogues of the invention act to a greater extent on the tissues of the liver than on those of the peripheral regions of the body,  
25       such as fat and muscle.

CLAIMS

1. The use of an analogue of a pharmaceutical active whose molecular weight is less than 25,000 Daltons in the manufacture of a composition for use in a method of treatment of the human or animal body, the analogue comprising a pharmaceutical active whose molecular weight is less than 25,000 Daltons covalently linked to a pendant molecular group wherein as a result of the administration of the composition to the human or animal body an active complex having a molecular weight of 25,000 Daltons or greater is present in the human or animal circulatory system.
2. Use according to claim 1 wherein the analogue is an insulin analogue and comprises an insulin or functional equivalent thereof covalently linked to a pendant molecular group wherein as a result of the administration of the composition to the human or animal body an insulin complex having a molecular weight of 25,000 Daltons or greater is present in the human or animal or circulatory system.
3. Use according to claim 1 or claim 2 wherein the pendant molecular group has an affinity for one or more binding proteins present in the human or animal circulatory system and wherein the total molecular weight of the pharmaceutical active and the pendant molecular group and the one or more binding proteins is 25,000 Daltons or greater.
4. Use according to claim 1 or claim 2 wherein the analogue also comprises one or more binding proteins non-covalently bound to the pendant molecular group, wherein the molecular weight of the analogue is 25,000 Daltons or greater.
5. Use according to any preceding claim, in which the pendant molecular group is based on a naturally occurring hormone or an analogue thereof.
6. Use according to any preceding claim, in which the pendant molecular group is an IGF1 group.

7. Use according to any preceding claim, in which the pendant molecular group is a thyroxyl group.
8. Use according to any preceding claim, in which the pendant molecular group is linked to the pharmaceutical active by a molecular spacer.
9. Use according to claim 8, in which the molecular spacer is a linear chain of from 3-10 carbon atoms.
10. Use according to claim 9, in which the molecular spacer is an aminohexanoyl group.
11. Use according to claim 10, in which the additional molecular group is a thyroxyl-aminohexanoyl group.
12. Use according to any of claims 3-5 or claim 7 or claim 11, in which the binding protein is albumin, thyroxine-binding pre-albumin or thyroxine-binding globulin.
13. Use according to any preceding claim, wherein the total molecular weight of the pharmaceutical active and the pendant molecular group is less than 25,000 Daltons, preferably less than 15,000 Daltons.
14. Use according to claim 1, wherein the total molecular weight of the pharmaceutical active and the covalently linked pendant molecular group is 25,000 Daltons or greater.
15. An analogue of a pharmaceutical active whose molecular weight is less than 25,000 Daltons comprising a pharmaceutical active whose molecular weight is less than 25,000 Daltons covalently linked to a pendant molecular group, said pendant molecular group having an affinity for one or more binding proteins present in the human or animal circulatory system.
16. An analogue according to claim 15 which is an insulin analogue and which comprises an insulin or functional equivalent thereof covalently linked to the pendant molecular group.
17. A preparation suitable for use in a method of treatment of the human or animal body, preferably suitable for subcutaneous injection, the preparation comprising at least one analogue of a pharmaceutical active whose

molecular weight is less than 25,000 Daltons comprising a pharmaceutical active whose molecular weight is less than 25,000 Daltons covalently linked to a pendant molecular group, said pendant molecular group having an affinity for one or more binding proteins present in the human or animal circulatory system.

18. A preparation according to claim 17 which is an insulin-based preparation which comprises at least one insulin analogue comprising an insulin or functional equivalent thereof covalently linked to a pendant molecular group, said pendant molecular group having an affinity for one or more binding proteins present in the human or animal circulatory system.

19. A method of insulin replacement therapy comprising subcutaneous injection of a preparation comprising at least one insulin analogue comprising an insulin or functional equivalent thereof covalently linked to an pendant molecular group, said pendant molecular group having an affinity for one or more binding proteins present in the human or animal circulatory system.

20. An analogue of a pharmaceutical active whose molecular weight is less than 25,000 Daltons comprising a pharmaceutical active whose molecular weight is less than 25,000 Daltons covalently linked to a large pendant molecular group, wherein the total molecular weight of the pharmaceutical active and the large pendant molecular group is 25,000 Daltons or greater.

21. An analogue of a pharmaceutical active whose molecular weight is less than 25,000 Daltons comprising a pharmaceutical active whose molecular weight is less than 25,000 Daltons covalently linked to a pendant molecular group, said pendant molecular group being non-covalently linked to one or more binding proteins.

22. An analogue according to claim 20 or claim 21 which is an insulin analogue and in which the pharmaceutical active is an insulin or functional equivalent thereof.

23. A preparation suitable for intravenous injection comprising at least one analogue according to claim 20 or claim 21.
- 5 24. A preparation according to claim 23 which is an insulin-based preparation, in which the analogue is an insulin analogue and the pharmaceutical active is an insulin or functional equivalent thereof.
- 10 25. A method of insulin replacement therapy comprising intravenous injection of a preparation according to claim 24.
26. A process for the synthesis of an analogue according to claim 15, wherein the pendant molecular group is a thyroxyl group, comprising the reaction of the pharmaceutical active with a thyroxyl compound.
- 15 27. An analogue according to claim 15 or claim 21 wherein the total molecular weight of the pharmaceutical active and the pendant molecular group is less than 25,000 Daltons, preferably less than 15,000 Daltons.
- 20 28. A preparation according to claim 18 or a method according to claim 19 wherein the total molecular weight of the insulin or functional equivalent thereof and the pendant molecular groups is less than 25,000 Daltons, preferably less than 15,000 Daltons.

## INTERNATIONAL SEARCH REPORT

Inv. No. PCT/GB 94/01784

## A. CLASSIFICATION OF SUBJECT MATTER

A 61 K 38/00, A 61 K 38/28

According to International Patent Classification (IPC) or to both national classification and IPC '6

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A 61 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP, A, 0 242 416 (VALCOR SCIENTIFIC LTD.) 28 October 1987 (28.10.87), claims 1,2,5,6,12,13, 15,16,22. -----	1,2, 15-25

☐ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the international search  
10 November 1994

Date of mailing of the international search report

06.12.94

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BÖHM e.h.



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 94/01784

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 19, 25  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 19, 25 are directed to a method of treatment of the human or animal body (Rule 39.1(iv)PCT) the search has been carried out and based on the alleged effects of the compounds.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

zum internationalen Recherchen-  
bericht über die internationale  
Patentanmeldung Nr.

to the International Search  
Report to the International Patent  
Application No.

au rapport de recherche inter-  
national relatif à la demande de brevet  
international n°

PCT/GB 94/01784 SAE 94565

In diesem Anhang sind die Mitglieder  
der Patentfamilien der im obenge-  
nannten internationalen Recherchenbericht  
angeführten Patentdokumente angegeben.  
Diese Angaben dienen nur zur Unter-  
richtung und erfolgen ohne Gewähr.

This Annex lists the patent family  
members relating to the patent documents  
cited in the above-mentioned inter-  
national search report. The Office is  
in no way liable for these particulars  
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of information.

La présente annexe indique les  
membres de la famille de brevets  
relatifs aux documents de brevets cités  
dans le rapport de recherche inter-  
national visée ci-dessus. Les renseigne-  
ments fournis sont donnés à titre indica-  
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im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
EP 242416		US A 4585754	29-04-86
		EP A1 242416	28-10-87
		AT E 65506	15-08-91
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		EP B1 242416	24-07-91